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STUDIES ON CO-PRECIPITATES OF MILK PROTEINS

3. FACTORS AFFECTING BEHAVIOUR IN AQUEOUS DISPERSIONS

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Summary

The effect of calcium content on some properties of co-precipitates in solution are considered. The solubility of the low-calcium type over a range of pH values was similar to casein, but 4% and 6% sodium tripolyphosphate was required for maximum solubility of medium- and high-calcium types. Some protein was insoluble at pH 7, and this appeared to be mainly aggregated whey protein and a sialic-acid-containing casein fraction. A colloid mill was required to disperse the granular form of co-precipitate, and homogenizing was necessary to produce almost sediment-free solutions from both granular and spray-dried forms.

Solutions of co-precipitates were "whitened" to approximately the same opacity as skim milk by the addition of $CaCl_2$ under controlled conditions, or by homogenizing with fat. Sodium tripolyphosphate appeared to stabilize these milk-like preparations towards heat sterilizing, but lactose decreased both their stability and whiteness.

Introduction

A method for the continuous production of co-precipitates of casein and whey proteins with controlled calcium contents was described in Part 1 of this series (Muller et al, 1967). The three types of co-precipitate that have been manufactured commercially have been described as "low", "medium" and "high" calcium, and they contain 0.5-0.8%, about 1.5%, and 2.5-3.0% calcium respectively. One of the aims in making co-precipitates with a range of calcium contents was to produce proteins with different functional properties. thereby extending the potential use of co-precepitate in a variety of food products with different viscosity, hydration, solubility and "whiteness' requirements. This paper describes some of the solubility and whitening properties of several forms of co-precipitate.

For some purposes, a more or less clear solution of the co-precipitate is desirable, whereas in other products, such as reconstituted milk-like drinks, a stable dispersion is required in order to impart a white, opaque appearance to the product.

Methods

(i) *Solubility*

The effect of pH and sodium tripolyphosphate (STPP) on the solubility of commercial samples of co-precipitate was examined by a method based on the standard solubility index method, Australian Standard N 61 — 1965. Protein-water mixtures were stirred in a Cenco Solubility Index Mixer for a standard time, filtered, and the protein remaining in solution in the filtrate determined. Modifications to the standard method for measuring the solubility of skim milk powder were as follows:—

(1) A 10 g sample of skim milk powder containing about 3.6 g protein is used for the standard test. Co-precipitate samples contained 82-86% protein, and 4.2 g was taken for the solubility test to give a mixture with protein content comparable with that used in the standard test.

(2) Sufficient NaOH or HCl was included with the distilled water before adding the sample to give the desired final pH after mixing.

(3) Instead of centrifuging, the solutions were filtered through a Whatman No. 541 filter paper. The percentage soluble protein was determined by Kjeldahl nitrogen values of the mixture before and after filtration.

(ii) *Dispersibility*

The dispersibility of spray dried co-precipitates at pH 7 was measured by the amount of Kjeldahl nitrogen remaining in the supernatant after centrifuging according to the standard solubility index method (500 g for 5 mins). This gave a measure of soluble protein plus finely dispersed material which remained in suspension after centrifuging.

(iii) *Solubility index*

This is expressed as volume in ml of sediment from 50 ml liquid, centrifuged as in (ii) above.

(iv) *Colour*

"Whiteness" of solutions was measured by the Agtron method, standardized against disc numbers 00 (Black) and 97 (Stark White).

TABLE 1
Solubility, dispersability and solubility index of s pray dried co-precipitate samples at pH 7, as defined in the text

Sample	Solubility (%)	Dispersability (%)	Solubility index
Low-Ca co-ppte	96	99	0.12
High-Ca co-ppte (6% STPP)	85	98	0.25
Sodium caseinate	99.5		<0.01
Skim milk powder	96		0.15

(v) *Heat stability test*

About 25 ml of co-precipitate solution in 1 oz McCartney bottles was kept in a 212°F bath for 5 min. and then transferred to a 248°F bath, and checked at 3 min. intervals for the appearance of precipitation or coagulation.

(vi) *Other analyses*

Sialic acid was determined by the method of Warren (1959), SH + SS by the pronase digestion method of Hill (1964), and calcium by the method of Dunkerley and Hayes (1967).

Results

(a) *Solubility:* Buchanan et al (1965) found that high-calcium co-precipitate, unlike casein, would not dissolve at pH 6-7 without the addition of a calcium-sequestering agent. Sodium tripolyphosphate was effective at levels of 4-6% of the weight of co-precipitate, depending on its calcium content. Preliminary experiments with low calcium co-precipitate showed that its solubility properties were similar to casein, and solutions were obtained by pH adjustment alone. Medium-calcium co-precipitates, however, required 2-4% STPP for maximum solubility at pH 6-7.

Solubility curves of 5% commercial spray-dried samples of high-calcium co-precipitate with 2% or 6% STPP added before spray drying, low-calcium co-precipitate, sodium caseinate and skim milk powder are shown in Fig. 1. No commercial spray-dried medium-calcium co-precipitate was available. It will be seen that all samples of co-precipitates were less soluble than caseinate or skim milk powder. High-calcium with 6% STPP

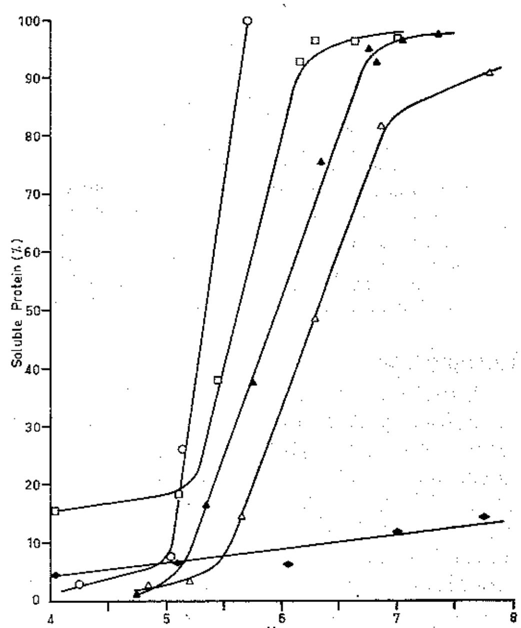

Fig. 1. The solubility of 5% spray dried sodium caseinate, skim milk and co-precipitate samples at various pH values, under conditions defined in the text.
○ sodium caseinate
□ skim milk powder
▲ low-Ca co-ppte
△ high-Ca co-ppte with 6% STPP
◆ high-Ca co-ppte with 2% STPP

TABLE 2
Effect of increasing shear on the solubility and solubility index of granular form co-precipitate samples at pH 7.

Sample	Colloid mill		Homogenizer	
	Solubility (%)	S.I.*	Solubility (%)	S.I.*
Low-Ca co-ppte	96	0.2	99.5	<0.01
Med.-Ca co-ppte (4% STPP)	95	0.2	99	<0.01
High-Ca co-ppte (6% STPP)	94	0.2	99	<0.01

*Solubility index, ml. sediment/50 ml. liquid centrifuged as described in text.

TABLE 3

Effect of number of passes through the colloid mill on the dispersability, solubility and solubility index of granular medium-calcium co-precipitate with 4% STPP at pH 7.

No. of passes through colloid mill	Dispersability (%)	Solubility (%)	Solubility Index*		Protein in total sediment (%)
			Total sediment	Heavy sediment	
1 (0.02″ gap)	89	83	3.5	1.5	11
2 (0.002″ gap)	93	84	3.0	1.0	7
3 „	95	84	2.5	0.5	5
4 „	95	85	2.4	0.5	5
5 „	94	86	2.5	0.25	6
6 „	95	87	2.0	0.1	5

*ml sediment/50 ml liquid centrifuged as described in text.

was less soluble than the low-calcium type. At pH 7, the solubilities of low-calcium co-precipitate and skim milk powder protein were both 96%, and high-calcium with 6% STPP was approximately 85%. The solubility of the latter increased to 92-95% at pH 8. The high-calcium with 2% STPP was the least soluble of all, 11% at pH 7 and 14% at pH 8.

(b) *Dispersibility:* As shown in Table 1, dispersibility values at pH 7 obtained for spray dried low-calcium co-precipitate and for high-calcium with 6% STPP were 99% and 98% respectively, compared with solubilities of 96% and 85%. The low-calcium product compared favourably with skim milk powder, but was less soluble than sodium caseinate (99.5%).

Some of the finely dispersed protein settled out of suspension on standing for several days and homogenizing was required to disperse this material to colloidal dimensions, as discussed below. High-calcium co-precipitate with 2% STPP did not form a stable suspension and settled out after standing for a short time.

Table 2 shows the effect of shearing forces on the solubility and solubility index values of the granular forms of co-precipitate at pH 7. Solutions treated in the PUC 7.5 H.P. colloid mill were prepared by adding granular co-precipitate to water at 170-190°F containing alkali and STPP. The mixture was circulated for 3 minutes, starting with the gap at 0.02″ and gradually reducing it to 0.002″, and then pumped out of the mill. A portion of these solutions was then given further work with higher shearing forces by homogenizing (Rannie) at 1,500 p.s.i. Prolonged circulation in the mill did not produce sediment-free solutions at pH 7, and these were obtained only after homogenizing, when the solubilities of all three types of co-precipitate reached 99%.

(c) *Commercial preparation of co-precipitate solutions:* Since spray-dried co-precipitates have good dispersibility in hot water, a colloid mill is not required and solutions with a low solubility index can be obtained by homogenizing alone. A colloid mill is, however, necessary for dispersing the granular form of co-precipitate before homogenizing. To study the efficiency of the colloid mill in dispersing the protein, a 5% suspension of granular medium-calcium co-precipitate containing STPP (4% on the weight of the co-precipitate) and a predetermined amount of NaOH to give a final pH of 7 and was stirred for 1 minute at 185°F and passed through the mill with the gap set at 0.02″. It was then passed several times through the mill with the gap at 0.002″ and the dispersibility, solubility and solubility index measured after each pass. As seen from Table 3, there was little change in either solubility or dispersibility after the second pass. At this stage the sediment was 7% of the total protein and packed in two layers in the centrifuge tube, with loosely packed material above a denser granular layer. There was no direct relationship between the amount of protein in the sediment and the solubility index.

(d) *Nature of the material insoluble at pH 7:* Under the conditions used for obtaining the solubility curves of spray-dried products in Fig. 1, approximately 15% of high-calcium co-precipitate with 6% STPP and 4% of low-calcium were insoluble at pH 7. There are several possiblities for the formation of less readily soluble material during manufacture of co-precipitate:

(i) Concentrations of $CaCl_2$ of approximately 2-3% at 40°C cause the soluble κ-casein complex to split off from sodium caseinate and the remainder of the casein forms an insoluble para-casein-like precipitate (Waugh and Von Hipple, 1956). Particles of insoluble material could thus be produced by high local concentrations of calcium chloride due to inadequate rate of mixing with the hot milk during the manufacture of medium- and high-calcium co-precipitates.

(ii) Flocs of denatured whey proteins might not have combined with the casein during the heat treatment of the milk but have been trapped in the precipitated casein curd before separation from the whey.

(iii) Scorched particles may have been derived from overheating during drying.

TABLE 4
Composition of co-precipitates and material insoluble at pH 7, compared with sodium caseinate and heat-coagulated whey proteins.

Material	Protein* (%)	SH + SS moles/10^5g protein	Sialic acid g/100g protein	Calcium g/100g protein
Sodium caseinate	99	3	0.35	0.003
Whey protein	85	19	0.36	0.07
Low-Ca co-ppte	93	7	0.34	0.6
Low-Ca co-ppte insol. at pH 7	81	16	0.56	0.1
Med.-Ca co-ppte†	92	6	0.32	1.6
Med.-Ca co-ppte insol. at pH 7	82	17	0.70	0.1
High-Ca co-ppte	89	5	0.31	3.5
High-Ca co-ppte insol. at pH 7	74	15	0.39	3.0

*dry weight basis.
†30 mesh granular form, dispersed in water at 180-190°F for 10 min. with an Ultra-turrax type T-45 high-shear mixer.

(iv) Particles of curd might not have been dissolved prior to spray drying.

An attempt was made to find the origin of this less readily soluble material by analysing its content of several constituents which are present in different amounts in whey proteins, casein and the insoluble paracasein-like material. If the material were mainly whey protein, then 17-30 moles SH + SS/10^5 gm would be expected and only trace quantities of sialic acid, which is absent in the major component, β-lactoglobulin. Casein has typical values of 1-1.3 moles SH + SS/10^5 gm and 0.4-0.5% sialic acid (Hill and Hansen, 1963). These values are considerably reduced in the paracasein-like material, since the SH and SS groups and sialic acid are present in the soluble κ-casein complex which is split off by treatment with $CaCl_2$. Furthermore, this precipitated material would be expected to have a higher calcium content than the casein from which it was derived.

Table 4 shows the values obtained for the three co-precipitates and for samples of the material insoluble at pH 7, and values for a commercial sample of sodium caseinate and denatured whey proteins (prepared by filtering the insoluble precipitate from acid whey heated to 180°F, pH 4.5).

Values for the SH + SS contents in the caseinate and whey protein preparation were 3 and 19 moles/10^5 respectively, 5-7 moles/10^5 g for the co-precipitates, and 15-17 moles/10^5 g protein in the material insoluble at pH 7. This suggests that the insoluble material was mainly whey protein and not an insoluble form of casein.

The sialic acid contents of the insoluble materials were in all cases greater than the co-precipitates from which they were derived, which shows clearly that the insoluble material was not κ-casein-depleted paracasein-like material. This is supported by the calcium contents, which were less than those of the parent co-precipitates. The presence of sialic acid in the whey protein preparation indicates that some of the casein fractions soluble at pH 4.5 had interacted and precipitated with the denatured whey proteins. This complex may be similar to that formed by a reaction between a sialic-acid-containing casein fraction and whey proteins which occurs under mild conditions and without heating to high temperatures, as reported by Beeby (1966).

The evidence is therefore consistent with the protein in the less soluble material being mainly denatured whey protein, and not scorched co-precipitate particles or some other form of casein. Protein and calcium accounted for 77-82% of the dry weight of the insoluble material. The remainder was not identified.

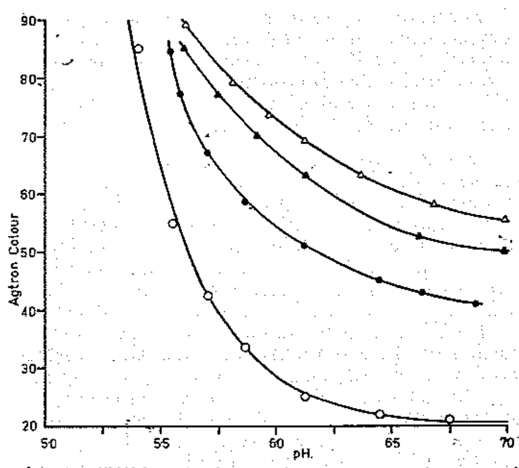

Fig. 2. "Whiteness" of 5% sodium caseinate and co-precipitate samples dissolved at pH 7 and adjusted to lower pH values.

○ sodium caseinate
▲ low-Ca co-ppte, spray dried
● medium-Ca co-ppte with 4% STPP, granular form
△ high-Ca co-ppte with 6% STPP, spray dried

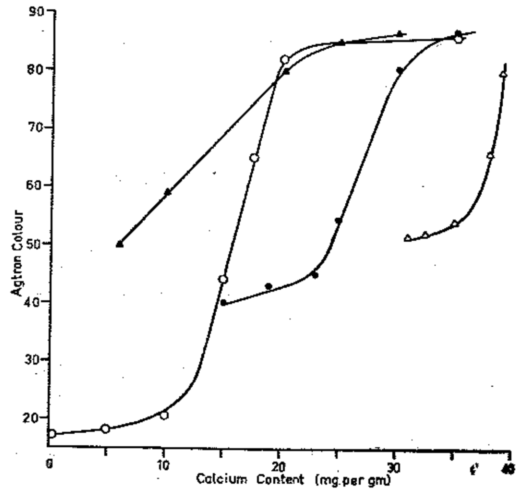

Fig. 3. The effect of calcium content on the "whiteness" of 5% sodium caseinate and co-precipitate samples at pH 7.
○ sodium caseinate
▲ low-Ca co-ppte, spray dried
● medium-Ca co-ppte with 4% STPP, granular form
△ high-Ca co-ppte with 6% STPP, spray dried

(e) *"Whiteness" of co-precipitate solutions:* The preparation of white milk-like colloidal suspensions of co-precipitate was attempted by decreasing the pH of the solution and by the addition of $CaCl_2$. The effect of fat on the opacity of co-precipitate solutions was also studied. A sample of commercial spray dried medium-calcium co-precipitate was not available when this work was carried out, and solutions of this material were obtained by dispersing commercial 30 mesh granular product in water containing 4% STPP on the weight of the co-precipitate at 180-190°F for 10 min. with an Ultra-turrax type T-45 high-shear mixer. Such high-shear treatment was necessary to disperse the granular form, since the rate of solution was much slower than the spray-dried product.

(i) *pH:* The effect of decreasing pH on the whiteness of 5% co-precipitate solutions dissolved at pH 7 is shown in Fig. 2. Although all three co-precipitate samples were whiter than sodium caseinate at the same pH, solutions with whiteness values corresponding to skim milk (80-90) were only achieved with high-calcium co-precipitate at pH values below 5.8, and with medium- and low-calcium at pH values below 5.6 and 5.7 respectively. None of these solutions had a heat stability greater than 3 min. and pH adjustment therefore appears to be an unsatisfactory method for increasing the whiteness of co-precipitate solutions.

(ii) *Ca content:* The effect of $CaCl_2$ on the whiteness of 5% co-precipitate solutions is shown in Fig. 3. Whiteness values corresponding to skim milk were achieved with sodium caseinate and low-calcium co-precipitate at calcium levels of 20 mg/g protein and at levels of 30 and 40 mg/g protein for medium- and high-calcium types respectively. The calcium levels in these suspensions

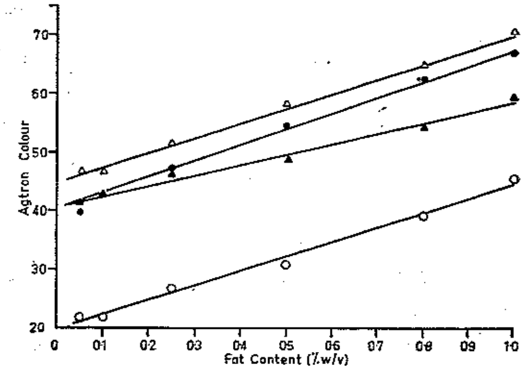

Fig. 4. The effect of fat on 4.2% sodium caseinate and co-precipitate samples at pH 7.
○ sodium caseinate
▲ low-Ca co-ppte, spray dried
● medium-Ca co-ppte with 4% STPP, granular form
△ high-Ca co-ppte with 6% STPP, spray dried

were similar to that in milk (approximately 35 mg Ca/g protein). For each type of co-precipitate, there was little visible difference in the whiteness of 5% and 10% solutions with the same calcium content per g protein.

A requirement for some milk-like drinks made from co-precipitate and lactose is that the protein should not precipitate when the product is sterilized at 248°F for 20 minutes. Table 5 shows the stabilizing effect of disodium hydrogen phosphate on the heat stabilities of 5% sodium caseinate and co-precipitate solutions, with and without lactose. In the series without added lactose, the heat stabilities of sodium caseinate, and medium- and high-calcium co-precipitates "whitened" with $CaCl_2$ were similar and met the required standard. The addition of $CaCl_2$ reduced the heat stability of low- and medium-calcium co-precipitates, although this effect was partly reversed by disodium hydrogen phosphate. The heat stabilty of low-calcium co-precipitate containing 20 mg Ca/g protein increased from 6-9 minutes to more than 30 minutes following the addition of 2% STPP, although this reduced the whiteness of the sample from 80 to 68. The effect of polyphosphates in stabilizing sterile concentrated milk has been reported by Leviton et al (1963) and Wilson et al (1963). Agtron values were not affected significantly by the heat treatment.

Heat stabilities were generally reduced in the series containing lactose. The Agtron values of mixtures which had heat stabilities of 30 min. fell to 39-50, compared with a value of 50 for skim milk heated for the same time and temperature.

(iii) *Fat content:* Fig. 4 shows the effect of safflower oil on the opacity of co-precipitate solutions after homogenizing at 2,500 + 350 p.s.i. and 25°C (Rannie). The Agtron colours of the fat-protein mixtures were measured after heating at 248°F for 20 minutes. The whiteness values of 4.2% high- and medium-calcium co-precipitate solutions containing 1% fat were both approximately 70. Low-calcium co-precipitate and sodium

TABLE 5

The effect of calcium, phosphate, STPP and lactose on the heat stability and "whiteness" of sodium caseinate and co-precipitate solutions, pH 7.

Sample	Protein (%)	Ca (mg/g protein)	Without lactose: Agtron Colour: Before heating	Without lactose: Agtron Colour: After heating 30 min.	Without lactose: Heat stability (min.)	With lactose: Agtron colour after heating‡	With lactose: Heat stability (min.)
Skim milk			83			50	>30
Sod. caseinate	5	0.03	17	16	>30		
" "	5	20	70	69	>30	42	12
" " + P	5	20	73	71	>30	50	>30
" "	10	0.03	21	20	>30		
" "	10	20	80		9–12		
Low-Ca co-ppte	5	6	50	49	>30		
" " "	5	20	80	81	6–9	67	<3
" " " + P	5	20	80	81	9–12	71	3–6
" " "	5	20	77		<3		<3
+ 0.5% STPP*	5	20	77		<3		
+ 1% STPP*	5	20	77		<3		<3
+ 2% STPP*	5	20	68	68	>30	44	>30
Low-Ca co-ppte	10	6	53	52	>30		
" " "	10	20	86		<3		
Med.-Ca co-ppte	5	15	40	40	>30		
" " "	5	30	69	68	25	48	>30
" " " + P	5	30	64	64	>30	39	>30
" " "	10	15	44	45	>30		
" " "	10	30	73	76	>30	50	20
High-Ca co-ppte	5	31	52	53	>30		
" " "	5	40	76	75	>30	51	15
" " " + P	5	40	72	71	>30	41	>30
" " "	10	31	55	54	>30		
" " "	10	40	77	78	>30	73	15

‡ Colour measured after heating at 248°F for times shown in "heat stability" column.

P Sample included 30 mg Na_2HPO_4/g co-precipitate.

* On the weight of co-precipitate medium-Ca contained 4% and high-Ca 6% STPP.

caseinate gave values of 60 and 45 respectively. These results suggest that when fat is included, less calcium would be required to whiten the mixture to a value corresponding to skim milk than when co-precipitate alone is used. Liquid fat should be sprayed into the protein-water mixture as it enters the colloid mill (Fig. 5).

Discussion

The results presented in this paper were obtained from one sample of each type of co-precipitate and they should therefore be considered only as indicative of the effects of calcium content on some of the properties of co-precipitates in solution. Differences in the properties could also arise from variation in the proportions of whey protein bound to the casein, in the amount of denatured whey protein trapped in the curd during precipitation, in the proportion of soluble casein components split off from the caseinate by treatment with heat, $CaCl_2$ and acid, in the pre-drying solubilizing treatment given to the spray dried forms, and in the severity of the drying conditions.

The results from the solubility tests suggests that solutions with low solubility index values could conveniently be prepared from granular co-precipitate on a commercial scale by soaking in hot water containing alkali and STPP (if required), circulated by a high-speed centrifugal pump to soften the curd (Fig. 5), and then after a few minutes diverting a proportion of the circulating suspension to the colloid mill. These stages are similar to the procedure of Burston et al (1967) for the manufacture of sodium caseinate from casein curd. The suspension should be filtered to protect the equipment before being homogenized. Material collected by the filter could be removed periodically and returned to the mixing vat for further treatment.

The results of the dispersion experiments suggest that the best approach to the manufacture of milk-like preparations from granular material would be to dissolve 5-10% co-precipitate as previously described, spray in $CaCl_2$ at (a) Fig. to adjust the Ca content of the co-precipitate to 20, 30 or 40 mg/gm for low-, medium- or high-

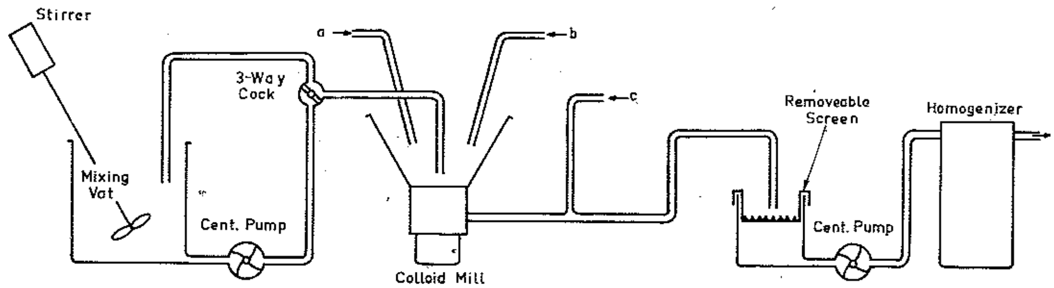

Fig. 5. Flow diagram showing equipment for the solution of co-precipitate.

calcium co-precipitates respectively, and simultaneously add sufficient alkali at (b) to adjust the pH of the suspension leaving the colloid mill to approximately 7. Adding the $CaCl_2$ and alkali in this way ensures thorough mixing and avoids local protein precipitation. The whitened co-precipitate is then filtered and homogenized. U.H.T. sterilizing instead of 20 minutes at 248°F would be desirable to minimise browning if the mixture contained lactose.

When starting with low-calcium co-precipitate, the 2% (on the weight of the protein) STPP is not added to the mixing vat but is sprayed in the line at (c) Fig. 5. The STPP is not required to solubilize this form of co-precipitate, and adding the STPP to the protein-$CaCl_2$ mixture gives a whiter product than adding the $CaCl_2$ to a protein-STPP mixture. Under the latter conditions, there is presumably a greater probability of the STPP sequestering the Ca rather than forming the desired Ca-protein complex.

The above recommendations should be considered only as guides to practical procedure since the details will depend on the equipment available and adjustments to the process may have to be made to compensate for variations in the characteristics of the particular co-precipitate. Only when the importance of these variations and their effects on products made from co-precipitate have been more fully established in commercial practise can realistic standards for the co-precipitates be formulated as a basis for control during their manufacture.

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